

FIG. 4. Water repellency of soil. Control flat left, treated flat right.

acids. Although a high contact angle (97°) is obtained for the partially hydrogenated tallow fatty acid-DETA reaction product, the percolation test still indicates inadequate hydrophobic properties. The completely hydrogenated tallow fatty acid-DETA reaction product, has a high contact angle and water fails to penetrate a bed of sand coated with this material even after a week.

A practical application of soil water repellents is shown in Figure 3. Soy seeds had been planted in treated soil and in an untreated control. After 4 weeks, only one seed in the treated soil germinated, as shown in the photograph. Figure 4 shows the two test flats after watering. Whereas the control flat held the water, the test flat permits the water to run around the soil and out through perforations in the bottom of the flat.

It has thus been shown that oxazolines and imidazolines derived from fats are useful intermediates for the synthesis of antibacterial agents and agricultural chemicals, respectively. It is hoped that the research reported here will further expand the utilization of fats and oils.

REFERENCES

1. Pfeffer, P.E., and L.S. Silbert, *J. Org. Chem.* 37:1256 (1972).
2. Linfield, W.M., R.A. Barauskas and F.O. Smith, *JAACS* 59:357 (1982).
3. Serota, S., J.B. Simon, E.B. Murray and W.M. Linfield, *J. Org. Chem.* 46:4147 (1981).
4. Linfield, W.M., T.J. Micich, T.J. Montville, J.R. Simon, E.B. Murray and R.G. Bistline Jr., *J. Med. Chem.* 26:Dec. (1983).
5. Jerchel, D., U.S. Patent 2,978,265 (1965).
6. Frump, J.A., *Chem. Rev.* 71:483 (1971).
7. Ackley, R.R., U.S. Patent 2,200,815 (1940).
8. Dobozy, O.K., *Tenside* 5:145 (1968).
9. Bistline, R.G., Jr., J.W. Hampson and W.M. Linfield, *JAACS* 60:823 (1983).

Reaction of Oxygen and Unsaturated Fatty Acids

F.D. GUNSTONE, Chemistry Department, The University, St. Andrew's KY16 9ST, Fife, Scotland

ABSTRACT

Oxygen reacts readily with unsaturated fatty acids so that every time these compounds are handled there is a danger they will become contaminated with oxidation products. The products formed first are allylic hydroperoxides which are labile molecules that change rapidly to other compounds, some of which are highly flavorful. Sometimes these changes are desirable and may be promoted; frequently they are not and have to be inhibited. Instrumental procedures recently introduced — especially separation by high performance liquid chromatography and identification by ^1H and ^{13}C nuclear magnetic resonance spectroscopy — have led to a renewed interest in this subject. For the nonenzymic processes of autoxidation and photooxygenation we now have a better understanding of the routes leading to the first-formed allylic hydroperoxides and an improved appreciation of the structure of further oxidation products including dihydroperoxides and hydroperoxides which also contain one or more cyclic peroxide units. Direct chemical routes to several of these compounds have also been developed. Oxidation of linoleic acid by plant-derived lipoxygenases gives diene hydroperoxides similar to those produced by autoxidation, except that the former are optically active and the latter racemic. Enzymic oxidation of arachidonic acid and certain related C_{20} acids in animal systems produces a wide variety of prostaglandins, thromboxanes and leukotrienes, all of which show interesting physiological properties. These compounds have been described as "tomorrow's drugs".

INTRODUCTION

The interaction of oxygen with unsaturated acids is an important reaction occurring under a wide range of conditions. Nonenzymic oxidation, occurring by autoxidation or photooxygenation, furnishes allylic hydroperoxides as

primary reaction products. These can be oxidized further to products which are now being identified. Recent advances have relied particularly on high performance liquid chromatography (HPLC) as an improved separation technique and on ^1H and ^{13}C nuclear magnetic resonance (NMR) spectroscopy and mass spectrometry for complete structure identification. Still further reaction leads to a wide range of compounds, including some of lower molecular weight associated with the development of flavor. This is sometimes desirable but frequently not so. Autoxidation and photooxygenation will be discussed and compared.

Enzymic oxidation, particularly in animal systems, is attracting considerable attention and the 1982 Nobel Prize for medicine was awarded to workers in this field. Such reactions are both regiospecific and stereospecific so that, compared with nonenzymic oxidation, the products of enzymic oxidation are often simpler in the number of components present although they may be more complex in structure. They are produced in enantiomeric form rather than as racemates. Many of the enzymic oxidation products have profound physiological properties with the curious observation that compounds which are structurally similar sometimes show antagonistic behavior. The possible involvement of lipid oxidation products in the cause of cancer, heart disease, asthma, arthritis, bronchial complaints and aging processes and also in their treatment, is a sufficient pointer to their importance.

AUTOXIDATION AND PHOTOOXYGENATION

Before oxygen and unsaturated fatty acids react nonenzymically, one of them must be activated. Either the

olefinic compound is converted to a resonance-stabilized allylic radical (as in autoxidation or some examples of photooxygenation) or the oxygen is raised to a more reactive singlet state (as in most photooxygenation reactions). These two reactions proceed by different mechanisms and give similar but nonidentical products.

Autoxidation

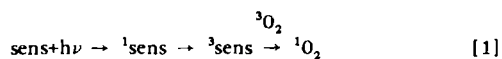
Autoxidation is a radical chain process involving initiation, propagation and termination (Scheme 1). The rate of reaction and the detailed structure of the hydroperoxides depend on the structure of the resonance-stabilized allylic radical $R\cdot$ produced from the unsaturated acid RH.

The exact nature of the initiation step is not fully understood, although it is known that initiation can be encouraged by suitable radicals, including those produced by a metal-catalyzed decomposition of preformed hydroperoxides. Hydroperoxides are frequently present in badly stored and badly handled oils and fats. It follows that, to inhibit autoxidation, the levels of prooxidant metals such as iron and copper should be kept to a minimum. Many of the synergists added to antioxidants to enhance their efficiency act as chelating agents. These include acids such as citric, ascorbic, phosphoric and ethylene diamine-tetraacetic acid.

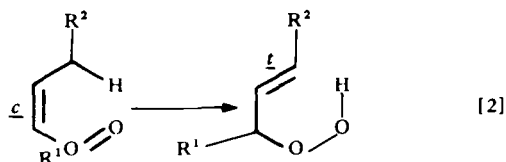
Antioxidants, on the other hand, shorten the length of the propagation sequence by enhancing the termination step. They are frequently phenolic compounds of natural (tocopherol) or synthetic origin (butylated hydroxyanisole, butylated hydroxytoluene, propyl gallate and tertiary butylhydroquinone). Only permitted compounds may be added to materials which are to be incorporated into human or animal food.

Photooxygenation

Photooxygenation requires singlet oxygen which is produced from the more usual triplet form of this element by interaction of light and a sensitizer such as chlorophyll, erythrosine, rose bengal or methylene blue:



Oxidation then proceeds by an ene reaction in which the singlet oxygen adds to an olefinic carbon atom with consequent migration of the double bond and change from *cis* to *trans* configuration.

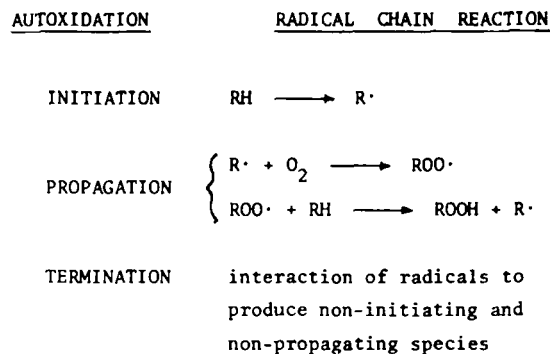


This reaction is unaffected by antioxidants but is inhibited by singlet oxygen quenchers such as carotene.

Photooxygenation is a much quicker reaction than autoxidation (Table I) and it has been suggested that the slower autoxidation reaction is initiated by hydroperoxides produced by photooxygenation made possible by traces of pigments left in the oil even after bleaching.

Monohydroperoxides

Autoxidation and photooxygenation proceed by different reaction mechanisms, occur at different rates, and produce slightly different compounds. Methyl oleate – a typical monoene – gives four hydroperoxides by autoxidation and only two by photooxygenation (Scheme 2). This is reversed with methyl linoleate: autoxidation gives two hydroperoxides, and photooxygenation furnishes four

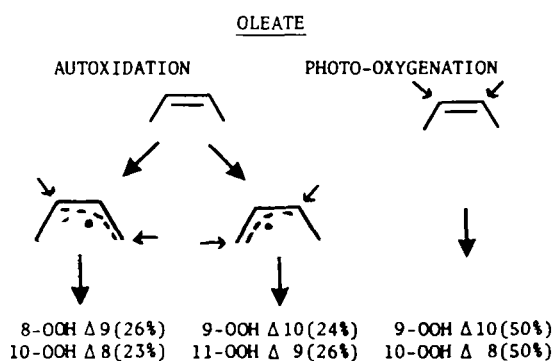


SCHEME 1

TABLE I

Relative Rates of Oxidation

	18:1	18:2	18:3
Autoxidation	1	27	77
Photooxygenation	30×10^3	40×10^3	70×10^3



SCHEME 2

(Scheme 3). These numbers are increased to four and six with methyl linolenate (Scheme 4), and to six and eight with methyl arachidonate. These numbers take no account of double bond configuration which will be discussed later.

Dihydroperoxides and Hydroperoxy Peroxides

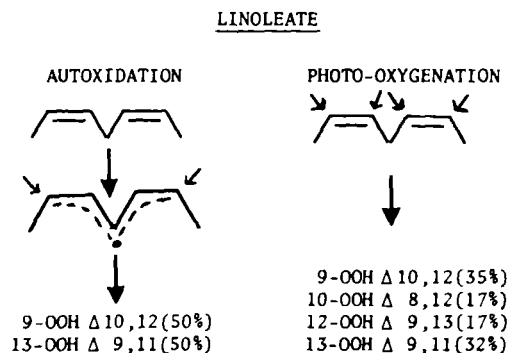
Unsaturated hydroperoxides can react with a second oxygen molecule to produce either dihydroperoxides or hydroperoxy cyclic peroxides. The former are produced from any hydroperoxide with an unsaturated system capable of further oxidation. In photooxygenation this means any nonconjugated double bond with room to migrate: in autoxidation an independent pentadiene unit is necessary.

For example, 9-OOH 18:3 (10*t*12*c*15*c*) yields the 9,12-, 9,15-, and 9,16-dihydroperoxides by photooxygenation, but only the 9,12- and 9,16-dihydroperoxides by autoxidation (Scheme 5). The partial structure in Scheme 5 also represents 16-OOH 18:3 (9*c*12*c*14*t*) and this behaves similarly. Dihydroperoxides are also produced from other monohydroperoxides on the basis set out in the previous paragraph.

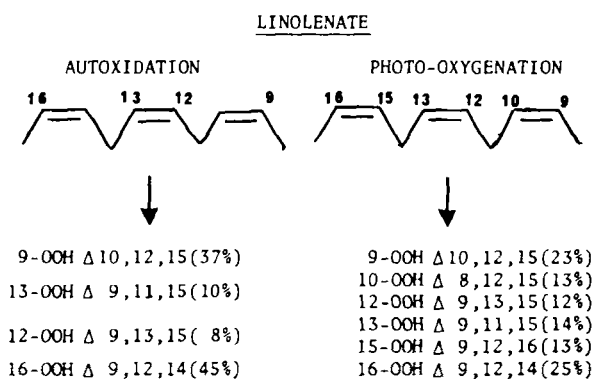
Hydroperoxy cyclic peroxides are a new kind of oxidation product obtained from hydroperoxides with a non-conjugated double bond in a β -position. Both mono- and

REACTION OF OXYGEN AND UNSATURATED FATTY ACIDS

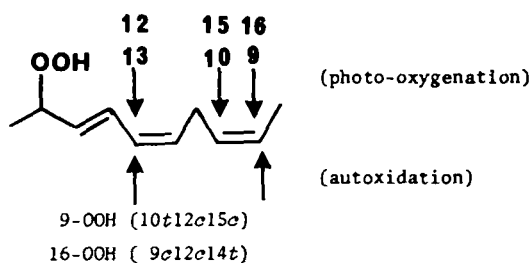
diperoxides have been identified from methyl linolenate (Schemes 6 and 7). Monoperoxides are also available from some linoleate hydroperoxides and the potential availability of these compounds is summarized in Scheme 8. They are more likely to be formed during photooxygenation, although they are produced during some autoxidation reactions.



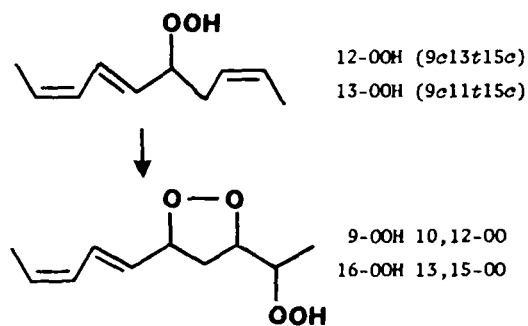
SCHEME 3



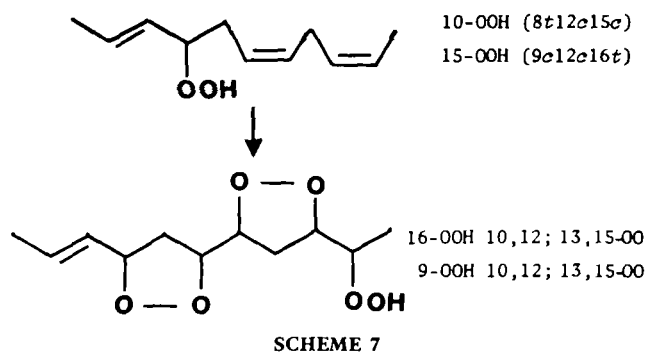
SCHEME 4

DIHYDROPEROXIDES

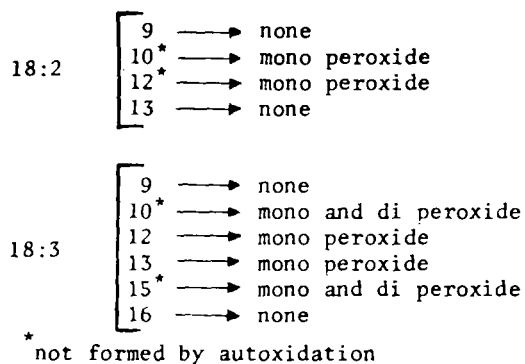
SCHEME 5

HYDROPEROXY PEROXIDES

SCHEME 6

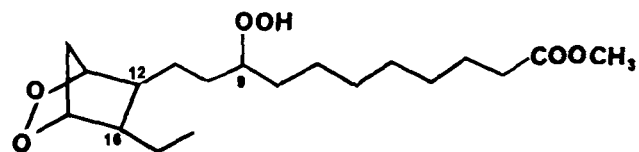
HYDROPEROXY DIPEROXIDES

SCHEME 7

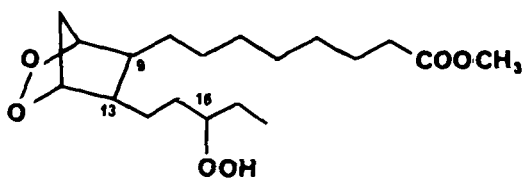
HYDROPEROXIDE → CYCLIC PEROXIDE

SCHEME 8

Yet other products include unsaturated acids with one or more epoxy, oxo or hydroxy functions and hydroperoxy peroxides such as 1 and 2 in which an additional carbon-carbon bond has been generated. These compounds are of special interest because of their prostaglandin-like structure. They are found among the products of photooxygenation of methyl linolenate.



1



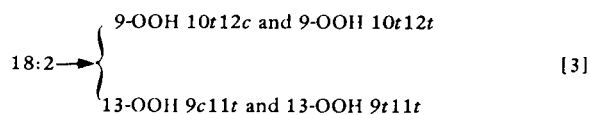
2

It is apparent from Schemes 2-4 that the various hydroperoxides are not formed in equal amounts: the yield of each "outer hydroperoxide" (9- and 13- from linoleate, 9- and 16- from linolenate) is greater than that of each "inner hydroperoxide" (10- and 12- from linoleate: 10-, 12-, 13- and 15- from linolenate). This has been explained in terms of the ease with which the latter undergo further oxidation to hydroperoxy peroxides.

Isomerization

Other aspects are interconversion of hydroperoxides and stereomutation of double bonds; in particular, conversion of conjugated *cis,trans* dienes to the all-*trans* form.

In the relatively simple case of linoleic acid, the diene hydroperoxides are a mixture of *cis,trans* and *trans,trans* isomers with the proportion of the latter increasing with reaction temperature (Eqn. 3). It has also been shown that individual hydroperoxides isolated from the reaction mixture are not stable and gradually revert to a mixture of the two hydroperoxides (Eqn. 4).



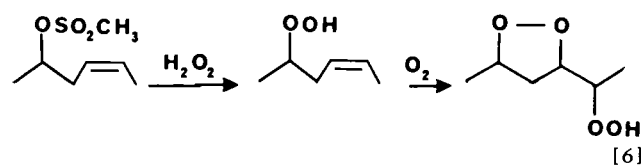
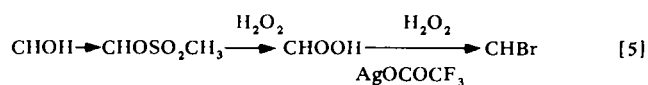
These changes have been interpreted in terms of the reversibility of hydroperoxide formation (Scheme 9) although these views have recently been questioned.

Studies in Norwich have demonstrated that the formation of *trans,trans* dienes and of cyclic peroxides is inhibited by α -tocopherol so that in the presence of a suitable amount of this compound autoxidation of linolenate, e.g., gives four hydroperoxides (all containing a *cis* double bond and a *c,t*-conjugated diene) in equal amount (compare Scheme 4).

Chemical Synthesis

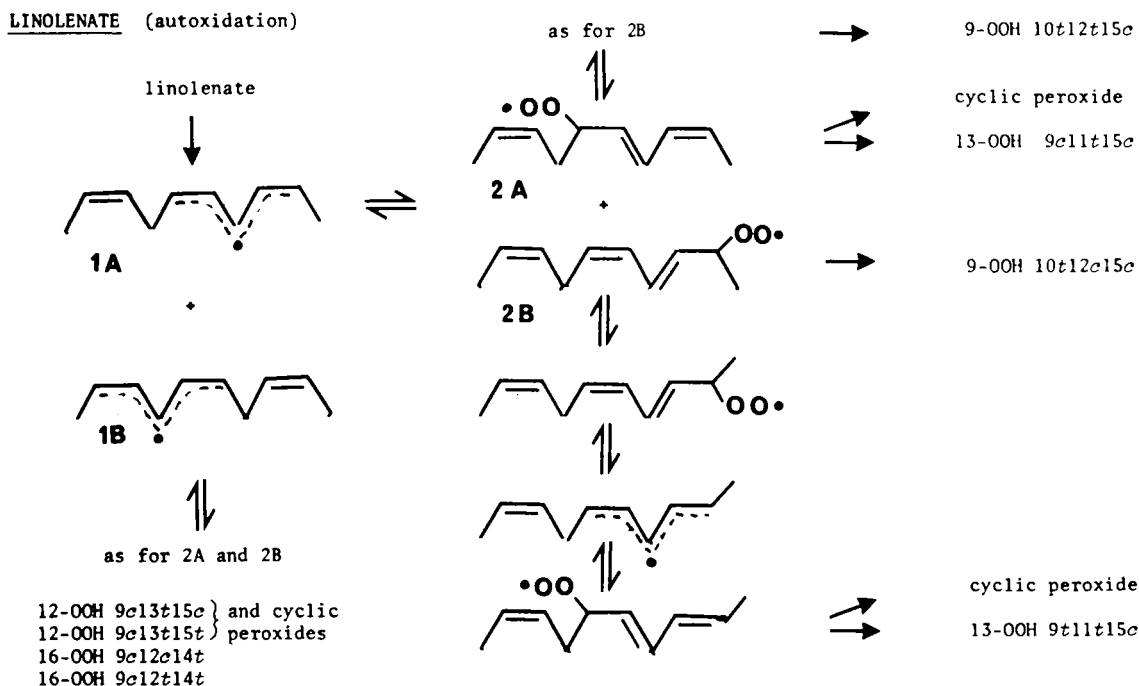
Attempts to prepare hydroperoxides and cyclic peroxides by direct chemical procedures leading perhaps to single products rather than mixtures have met with only limited success. Improvements in these procedures are to be desired.

Hydroperoxides can be made from methanesulfonates and hydrogen peroxide or from bromides and hydrogen peroxide in the presence of a silver salt (Eqn. 5). Reaction with the methanesulfonate from ricinoleate (Eqn. 6) is complicated by further reactions leading to cyclic peroxides and to cyclopropane compounds.



Allylic hydroperoxides (such as the 1:1 mixture of 9-OOH $\Delta 10t$ and 10-OOH $\Delta 8t$ available from methyl oleate by photooxygenation) have been converted to cyclic peroxides by two routes. In each case the product is a mixture of the 8,10- and 9,11-peroxides (Scheme 10).

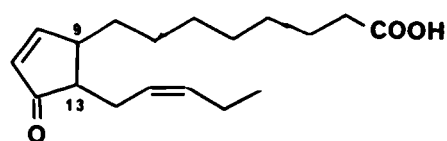
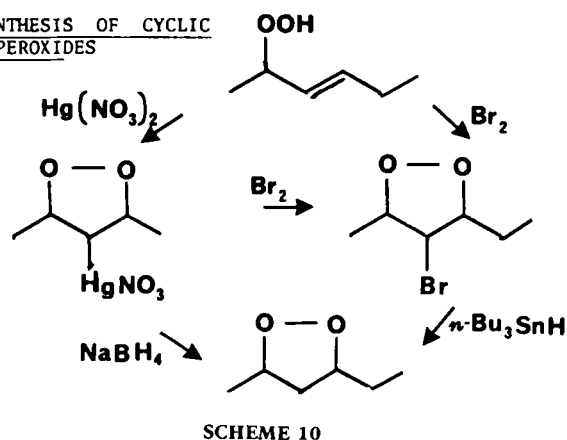
Conjugated dienes (such as 18:2 9t11t) react with singlet oxygen to furnish an unsaturated 6-membered cyclic peroxide. The double bond can be smoothly epoxidized or brominated but it has not proved possible to reduce it to the saturated peroxide. The peroxide readily gives a furan under a range of conditions (Scheme 11).



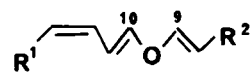
SCHEME 9

REACTION OF OXYGEN AND UNSATURATED FATTY ACIDS

SYNTHESIS OF CYCLIC PEROXIDES



4

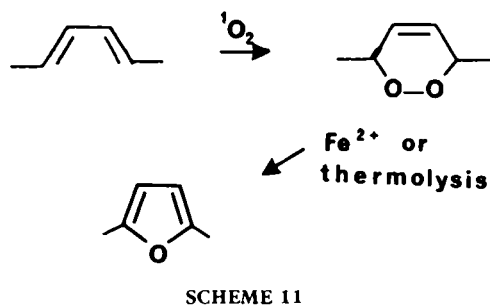


from 18:2 (potato)

 $R^1 = \text{CH}_3(\text{CH}_2)_4$ $R^2 = (\text{CH}_2)_6\text{COOH}$

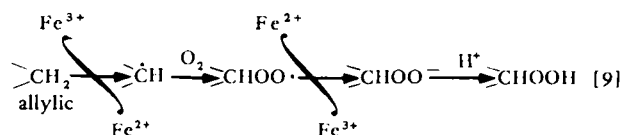
5

SYNTHESIS OF CYCLIC PEROXIDES



The flaxseed product is interesting because of its resemblance to prostaglandins, and in the potato product oxygen has been inserted between C-9 and C-10.

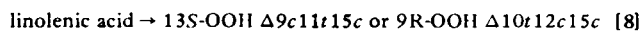
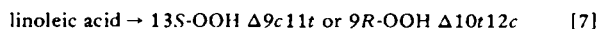
Lipoxygenases contain iron and hydroperoxidation probably occurs through the following series of one electron changes:



ENZYMIC OXIDATION

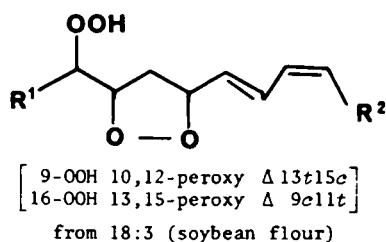
Plant-Derived Lipoxygenases

Oxidation of polyene acids under the influence of plant-derived lipoxygenases has been studied extensively. The reaction is probably regiospecific and stereospecific but enzyme preparations are not always pure. Consequently, mixed hydroperoxides are sometimes obtained or a second enzymic process leads to a modified product.

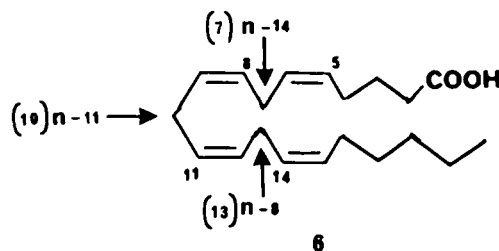


The 9-hydroperoxides are conveniently prepared using a lipoxygenase preparation from potato or tomato, whereas the 13-hydroperoxide is best prepared with a lipoxygenase preparation from soybean.

Less conventional products which have been identified include compounds 3-5 using in each case the materials indicated.



3



Appropriate lipoxygenases promote the stereospecific formation of hydroperoxides at C(5), C(11), C(12) and C(15), requiring removal of hydrogen from C(7)(n-14), C(13)(n-8), C(10)(n-11) and C(13)(n-8), respectively. Thus each of the doubly allylic methylene groups may be involved in these processes. Of these, the most significant is the 5S-hydroperoxide ($\Delta 6t8c11c14c$) which is the precursor of the epoxide (LTA4) and the leukotriene (LTC4). This last was previously recognized as the "slow reacting substance of anaphylaxis" and is a causative agent in asthma.

Research groups in many laboratories have endeavored — with success — to synthesize these compounds and others with related structures in the hope that these would also display interesting physiological properties.

A natural prostaglandin (PGA₂) discovered in the gorgonian coral (*Plexaura homomalla*) can be isolated easily and converted to other prostaglandins. Some entirely new prostaglandins (clavulones or clavaridenone) have been isolated recently by Japanese workers from another marine source.

In the early 1940s E.H. Farmer and his colleagues, studying the oxidation of natural rubber, examined the reaction of methyl oleate and linoleate in the belief that their oxidation might be simpler than that of rubber. In 1943 they reported, for the first time, that these unsaturated esters furnished olefinic hydroperoxides. Their ideas were considered to be novel and challenging since the general view at that time was that oxidation must be accompanied by disappearance of the olefinic center.

Forty years ago (August 1943) I began my Ph.D studies in the University of Liverpool with T.P. Hilditch. Part of

my research project was concerned with oxidation of methyl oleate and linoleate. Radical reactions were not then fully understood. Developments in the past few years have shown that we did not solve all the problems of oxidation in the 1940s. There are probably more surprises to come.

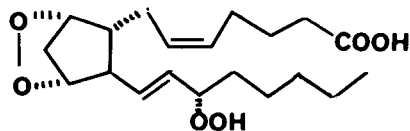
REFERENCES

Methyl oleate — autoxidation

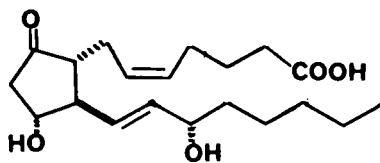
1. Frankel, E.N., W.E. Neff, W.K. Rohwedder, B.P.S. Khambay, R.F. Garwood and B.C.L. Weedon, *Lipids* 12:901 (1977).
2. Neff, W.E., E.N. Frankel, C.R. Scholfield and D. Weisleder, *Lipids* 13:415 (1978).
3. Neff, W.E., and E.N. Frankel, *Lipids* 15:587 (1980).
4. Frankel, E.N., *Prog. Lipid Res.* 19:1 (1980).

Methyl oleate photooxygenation (see also references 3 and 4)

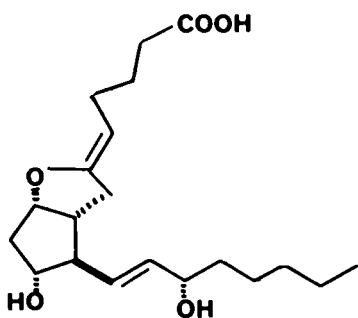
5. Chan, H.W-S., *JAOCS* 54:100 (1977).
6. Terao, J., and S. Matsushita, *JAOCS* 54:234 (1977).
7. Frankel, E.N., W.E. Neff and T.R. Bessler, *Lipids* 14:961 (1979).



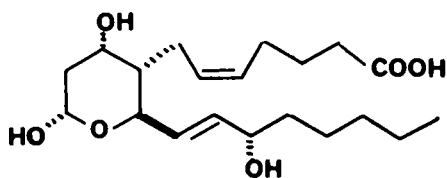
PGG₂



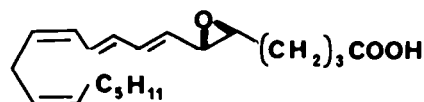
PGE₂



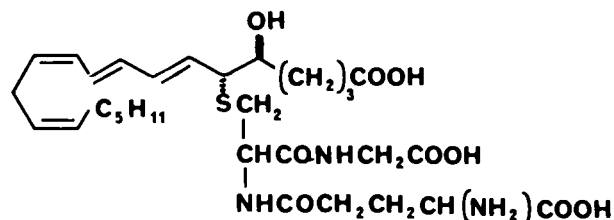
PGI₂



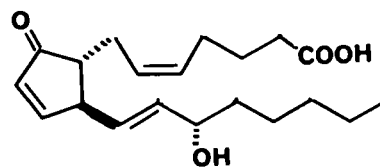
TXB₂



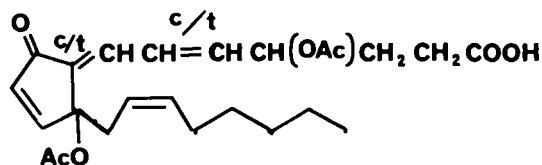
LTA₄



LTC₄



PGA₂



CLAVULONES

Methyl linoleate – attoxidation (see also references 2-4).

8. Chan, H.W-S. and G. Levetz, *Lipids*, 12, 99 (1977).
9. Frankel, E.N., W.E. Neff, W.K. Rohwedder, B.P.S. Khambay, R.F. Garwood and B.C.L. Weedon, *Lipids* 12:908 (1977).
10. Peers, K.E., D.T. Coxon and H.W-S. Chan, *J. Sci. Food Agric.* 32:898 (1981).
11. Porter, N.A., B.A. Weber, H. Weenen and J.A. Khan, *J. Am. Chem. Soc.* 102:5597 (1980).
12. Porter, N.A., L.S. Lehman, B.A. Weber and K.J. Smith *Ibid.* 103:6447 (1981).

Methyl linoleate – photooxygenation (see also references 3, 4, 6 and 7)

13. Thomas, M.J., and W.A. Pryor, *Lipids*, 15, 544 (1980).
14. Mihelich, E.D., *J. Am. Chem. Soc.* 102:7141 (1980).
15. Frankel, E.N., W.E. Neff, E. Selke and D. Weisleder, *Lipids* 17:11 (1982).

Methyl linolenate – autoxidation (see also references 3, 4 and 10)

16. Frankel, E.N., W.E. Neff, W.K. Rohwedder, B.P.S. Khambay, R.F. Garwood and B.C.L. Weedon, *Lipids* 12:1055 (1977).
17. Chan, H.W-S., J.A. Matthew and D.T. Coxon, *Chem. Commun.* 235 (1980).
18. Neff, W.E., E.N. Frankel and D. Weisleder, *Lipids* 16:439 (1981).

Methyl linolenate – photooxygenation (see also references 3-7)

19. O'Connor, D.E., E.D. Mihelich and M.C. Coleman, *J. Am. Chem. Soc.* 103:223 (1981).
20. Coxon, D.T., K.R. Price and H.W-S. Chan, *Chem. Phys. Lipids* 28:365 (1981).
21. Neff, W.E., E.N. Frankel and D. Weisleder, *Lipids* 17:780 (1982).
22. Frankel, E.N., W.E. Neff and D. Weisleder, *Chem. Commun.* 599 (1982).

Chemical synthesis

23. Corey, E.J., A. Marfat, J.R. Falck and J.O. Albright, *J. Am. Chem. Soc.* 102:1433 (1980).
24. Corey, E.J., J.O. Albright, A.E. Barton and S. Hashimoto, *Ibid.* 102:1435 (1980).
25. Porter, N.A., B.A. Weber, H. Weenen and J.A. Khan, *Ibid.* 102:5597 (1980).
26. Porter, N.A., D.H. Roberts and C.B. Zeigler Jr., *Ibid.* 102:5912 (1980).
27. Porter, N.A., A.N. Roe and A.T. McPhail, *Ibid.* 102:7574 (1980).
28. Frankel, E.N., D. Weisleder and W.E. Neff, *Chem. Commun.* 766 (1981).
29. Bascetta, E., Ph.D. thesis, University of St. Andrews, 1983.

Enzymic oxidation – plant systems

30. Veldink, G.A., J.F.G. Vliegthart and J. Boldingh, *Prog. Chem. Fats Other Lipids* 15:131 (1977).
31. Zimmerman, D.C., and P. Feng, *Lipids* 13:313 (1978).
32. Vick, B.A., P. Feng and D.C. Zimmerman, *Lipids* 15:468 (1980).
33. Feng, P., B.A. Vick and D.C. Zimmerman, *Lipids* 16:377 (1981).
34. Van Os, C.P.A., G.P.M. Rijke-Schilder, H. van Halbeek, J. Verhagen and J.F.G. Vliegthart, *Biochim. Biophys. Acta*, 663:177 (1981).

Enzymic oxidation – animal systems

35. Nelson, N.A., R.C. Kelly and R.A. Johnson, *Chem. Eng. News*, 16 Aug.:30 (1982).
36. Ackroyd, J., and F. Scheinmann, *Chem. Soc. Rev.* 11:321 (1982).
37. Kikuchi, M., and Y. Tsukitani, *Tetrahedron Lett.* 23:5171 (1982); Kobayashi, M., T. Yasuzawa, M. Yoshihara, H. Akutsu, Y. Kyogoku and I. Kitigawa, *Ibid.* 23:5331 (1982).

Microbial Conversions of Alkanes and Fatty Acids

COLIN RATLEDGE, Department of Biochemistry, University of Hull, Hull, HU6 7RX, United Kingdom

ABSTRACT

Alkanes are attacked readily by a wide variety of microorganisms. The most frequently encountered mode of oxidation is for one of the terminal methyl groups to be oxidized, through the alkanol, then the alkanal, to the corresponding fatty acid. Alkanes may be attacked subterminally also, and various ketones as well as the corresponding secondary alcohols can be produced. Subsequent degradation of these ketones occurs via introduction of oxygen into the chain to give a corresponding ester, which is then hydrolyzed to give a primary alkanol 2 carbon atoms shorter than the original alkane. The fatty acids arising by either route of oxidation, or by gratuitous introduction to the microbial system, may be oxidized by: (a) β -oxidation to give a number of acetyl-CoA units—intermediates of the process cannot be isolated from this pathway due to the tightly coupled nature of the substrates to the enzymes; (b) α -oxidation; or (c) oxidation at the other end of the molecule. In the latter case, ω - and $\omega-1$ -hydroxyfatty acids can be produced. ω -Hydroxyfatty acids are subsequently oxidized to give dicarboxylic acids, which can be isolated, sometimes in high yield, by use of appropriate microbial mutants lacking in certain of the key metabolizing enzymes. With some yeasts, the fatty acids, including the ω -hydroxyfatty acids, can be esterified to various sugars to give a series of glycolipids. In some cases, wax esters are formed between fatty acid and alkanol; these wax esters can include diunsaturated molecules having a close chemical similarity to those of sperm whale and jojoba oils. Various recent innovations have occurred using isolated enzyme systems which can be used in transesterification reactions to convert cheap triacylglycerols into high value added commodities such as cocoa butter.

INTRODUCTION

Microorganisms, i.e., bacteria, yeasts and molds, can grow on a wide variety of hydrocarbons as sole sources of carbon and energy. They can partially oxidize an even greater range of such compounds. The list of compounds attacked is extensive and includes straight and branched chain alkanes, alkenes, alicyclic, heterocyclic and aromatic hydrocarbons. Indeed, there are probably few compounds that cannot be attacked, at least partially, by some microorganism; the most recalcitrant molecules are probably the macromolecular polymers such as polyethylene and polystyrene, where there are considerable difficulties for the microorganism to produce a solubilizing enzyme prior to oxidative degradation. Of course, there is no single organism which will utilize all hydrocarbons but, in general, each organism can utilize a range of hydrocarbons as sole source of carbon and energy.

The most readily assimilated hydrocarbons are the straight chain alkanes from C₁₀ to C₁₈. Utilization of long-chain alkanes, e.g., plant paraffins of up to C₃₅, is less widespread, but some examples, particularly amongst the bacteria, have been reported (1). Isoalkanes with a single methyl side chain can be utilized for growth and, like the straight chain alkanes, be incorporated into cell components such as lipids. However, isoalkanes with branched chains at both ends of the molecule tend not to be utilized